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Review

# Genetic and biochemical intricacy shapes mitochondrial cytopathies

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ABSTRACT

The major progress made in the identification of the molecular bases of mitochondrial disease has revealed the huge diversity of their origin. Today up to 300 mutations were identified in the mitochondrial genome and about 200 nuclear genes are possibly mutated. In this review, we highlight a number of features specific to mitochondria which possibly participate in the complexity of these diseases. These features include both the complexity of mitochondrial genetics and the multiplicity of the roles ensured by the organelles in numerous aspects of cell life and death. This spectacular complexity presumably accounts for the present lack of an efficient therapy in the vast majority of cases.



**Contents**



## Introduction

Considering the crucial role of mitochondria in energy production and the implication of these organelles in major cell metabolic pathways, it seems intuitive that any significant mitochondrial dysfunction should be either lethal or at least bring quite severe developmental anomalies, particularly in the brain. Accordingly, fetal lethality is early seen upon genetic disruption of genes encoding key mitochondrial proteins in mammalian models. In the mouse, lethality due to mitochondrial defect occurs at about day 8.0 of embryogenesis

*Abbreviations:* Aif, apoptosis-inducing factor; AOX, alternative oxidase; α-KGDH, α-ketoglutarate dehydrogenase; mtDNA, mitochondrial DNA; Nd1, internal NADH dehydrogenase (*Saccharomyces cerevisiae*, mitochondrial); PG, propyl gallate; PGC1, peroxisome proliferator-activated receptor gamma coactivator 1; PGL, paraganglioma; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; SDH, succinate dehydrogenase; tRNA, transfer RNA; TCAC, tricarboxylic acid cycle

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(E8.0; equivalent in humans to Carnegie stage 8; day 17–19) (Larsson and Rustin, 2001). Prior to this, anaerobic (glycolytic) metabolism is regarded as the essential source of ATP necessary for embryonic development (Baker and Ebert, 2013). After this point, a shift to aerobic (oxidative phosphorylation) metabolism favored by increased oxygen supply, the so-called fetal shift, would sustain the organogenesis taking place during fetal development and later on.

The actual frequency of very early fetal lethality originating from mitochondrial dysfunction is difficult to estimate in humans. Yet, we know that severe mitochondrial dysfunction from genetic origin, although relatively rare, can occur at various ages in humans (Munnich and Rustin, 2001). This presupposes either a successful passage through the fetal period despite defective mitochondria, or a delayed appearance of the mitochondrial dysfunction after early fetal life. Subjects of this review, the complexity of mitochondrial genetics and the multiplicity of the roles ensured by the organelles, might provide explanations or rational hypotheses for these surprising observations.

## Mitochondrial DNA genetics

In mammals, about 1500 proteins are required to build and ensure the functioning of mitochondria (Lopez et al., 2000). More than 99% of these are encoded by nuclear DNA, being spread on the different chromosomes, including the X chromosome. However, mitochondria, as chloroplasts in plant, possess their own DNA (mtDNA) resulting in an unorthodox genetics (Larsson and Clayton, 1995). In humans, except for extremely rare aberrant cases, strict maternal inheritance of mtDNA is observed (Taylor et al., 2003). The mitochondria from sperm are tagged with ubiquitin for elimination after fecundation (Sato and Sato, 2013). Due to this complex genetics with both nuclear and mitochondrial genomes involved, transmission of mitochondrial traits, including pathological ones, will thus follow different types of inheritance possibly known in humans: autosomal, X-linked, or maternal inheritance (Zeviani et al., 1989).

The intron-less 37 genes harbored by the circular mtDNA (16,569 bp) in humans encode 13 subunits of the respiratory chain, 22 transfer RNAs and 2 ribosomal RNAs (Fig. 1) (Attardi et al., 1989). Each cell possesses a high number of mitochondria, constantly undergoing fusion and fission, and even more mitochondrial DNA molecules. Thus several hundreds of thousands of copies of mtDNA, contained in several tens of thousands of mitochondria, are found in human oocytes, albeit with significant disparities between oocytes from one woman (Fig. 2). The huge pool of mitochondria contained in the fertilized egg is then randomly distributed between daughter cells. Through the successive divisions, mtDNA is thus progressively reduced to a thousand or less, a number varying according to cell types. But even after this drastic reduction in the number of mtDNA molecules, there is still plenty of room for heterogeneity in the mtDNA population in each cell. Indeed, cells will easily withstand a low-noise of deleterious base changes in mitochondrial mtDNA and together with mtDNA polymorphisms transmit it to daughter cells. This is presumably due to the low selection pressure resulting from the occurrence of high number of mtDNA copies and the limited functional engagement of the mitochondrial oxidative metabolism under most conditions. Noticeably, most of the huge population of mitochondria of the oocytes is presumably at rest as indicated by the absence of well-formed cristae, folds in the inner membrane which carry the respiratory chain complexes. As a result, oocytes will tolerate mtDNA mutations that might be deleterious at later stages. Interestingly, an excess of mitochondrial capacity under non-stressing condition has been also reported in the skeletal muscle of mouse where mitochondrial function has been impaired by the double knockout of the gene encoding PGC1α and β (Rowe et al., 2013). Obviously, the variation of this over capacity according to tissues, cell types, conditions or age, may participate in the striking tissue expression and course observed in most patients with mitochondrial diseases.

Within the mtDNA population of a cell, base changes may be either harbored by all mtDNA molecules of a cell, a condition known as homoplasmy, or only found in a sub-population of these mtDNA molecules, a condition known as heteroplasmy. The tissue-specific level of heteroplasmy in terms of functional mtDNA (low content) has been shown to mirror tissue expression of disease in a number of patients (Ballana et al., 2008). Incidentally, mixing or segregation of mtDNA polymorphisms through the strict maternal transmission provides a

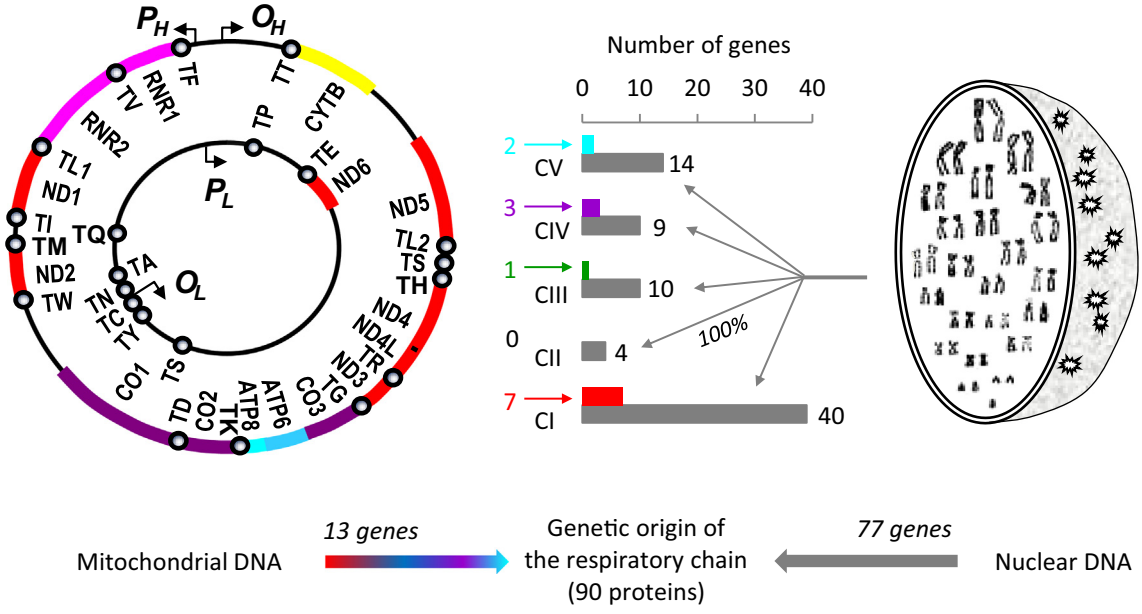


**Fig. 1.** Genes encoding mitochondrial respiratory chain subunits. The scheme shows the dual origin of the components of the respiratory chain encoded either by genes harbored by the mitochondrial DNA (left) or by genes distributed on the 46 (2 x 23) chromosomes of the nucleus (right). The medium graph specifies the nuclear (grey) or the mitochondrial (color) origin of the sub-units for each respiratory chain complex. Specifically, the four subunits constitutive of Complex II are all encoded by nuclear , *B, C*, and D. An identical color code is used for the scheme mapping the genes on the mitochondrial DNA. The displacement loop (D-loop) on the mitochondrial DNA map contains sequences that are used to initiate both mtDNA replication and transcription, including the L- and H-strand promoters (PL and PH, respectively) and the origin of H-strand replication (OH). The origin of light-strand replication is indicated (OL). Grey circles denote the different mitochondrial tRNAs harbored by the mitochondrial DNA.

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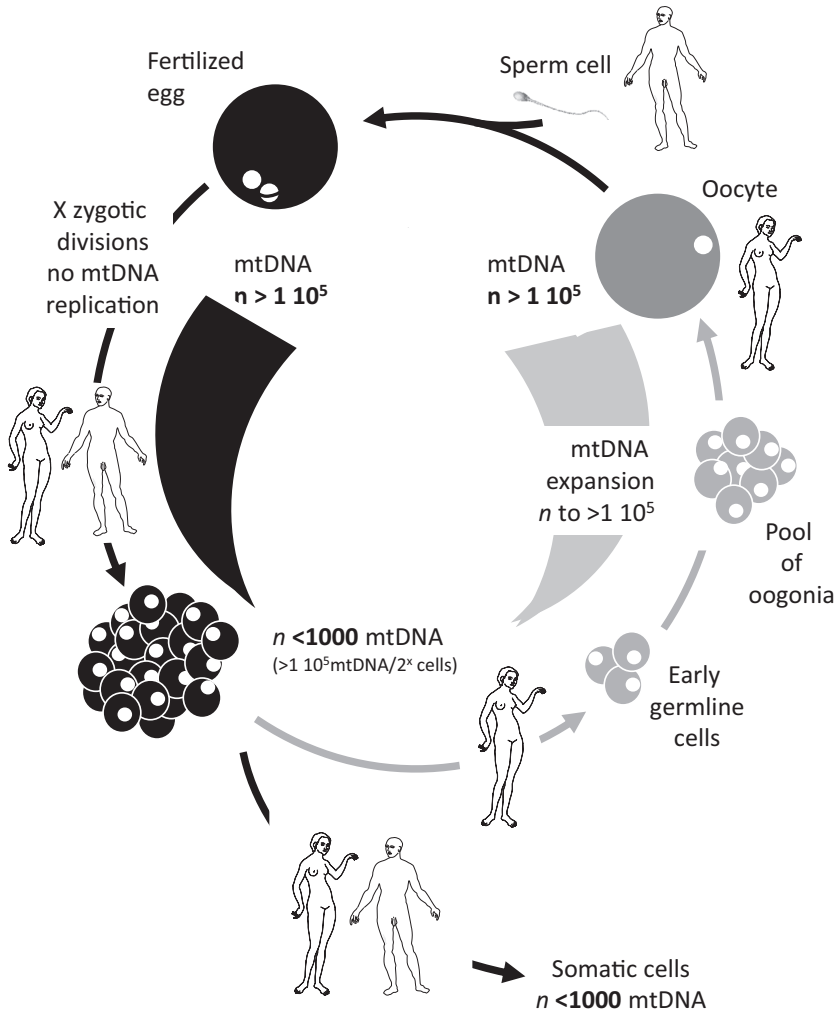


**Fig. 2.** Flow chart showing the changes in the number of mitochondrial DNA molecules during the reproduction cycle in humans. There is a huge number of mitochondria present in mature oocytes (top left), the mitochondria at this point may occupy more than 50% of cellular volume. After fecundation, a drastic reduction of this number occurs ultimately resulting in less than a thousand mitochondria DNA molecules per somatic cells.

unique opportunity to study human population genetics and migration (Kenney et al., 2014).

## The nuclear side of mitochondria

Most of the genetic material initially contained in the ancestral microorganism at the origin of mitochondria of modern cells is now spread in the different chromosomes of the human genomes, including a large number of pseudo-copies for most gene encoding respiratory chain components (Rustin et al., 2007). Indeed, apart from the 13 respiratory chain components still coded by the mtDNA and the machinery utilized for the transcription of these, all mitochondrial components are nuclearly encoded (Fig. 1). So far mutations in more than 150 nuclear genes have been shown to directly or indirectly impede mitochondrial functioning, a list of these genes and their mutations is regularly updated at *MitoMap* (Ruiz-Pesini et al., 2007). According to *MitoPhenome* review about 500 phenotypic features have been associated with mutations in 174 genes (Scharfe et al., 2009). As a result, today most medical specialties could see patients with mitochondrial disease (Table 1). It can be predicted that with improved methods and lower costs for gene mutation screening, the spectrum of diseases with recognized mitochondrial origin will further widen (Taylor et al., 2014).

Quite surprisingly, the diseases associated with mutations in nuclear genes encoding mitochondrial proteins, their course, their tissue-specific expression, reveal as difficult to understand that those originating from comprehensibly complex heteroplasmic mtDNA mutations (Briere et al., 2004). An illustration of this complexity is given by mutations affecting the genes encoding respiratory-chain complex II

**Table 1**
Clinical entities concerned by primary genetic mitochondrial disorders.
Clinical entities are classified and phenotypes collected for each mitochondrial disease-causing gene according to MitoPhenome (http://www.mitophenome.org/) (Ruiz-Pesini et al., 2007).

| Clinical categories | Number of distinct phenotypic entities (502 total) |
|---|---|
| Neurologic | 93 |
| Metabolic | 72 |
| Oncologic | 39 |
| Endocrinologic | 34 |
| Ophthalmologic | 32 |
| Hematologic | 29 |
| Gastrointestinal | 28 |
| Musculoskeletal | 27 |
| Genitourinary | 24 |
| Dermatologic | 23 |
| Psychiatric | 21 |
| Cardiovascular | 18 |
| Respiratory | 13 |
| Immunologic | 5 |
| Miscellaneous | 44 |

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(succinate dehydrogenase; SDH) which all result, albeit with a variable severity, in a decreased activity of the enzyme. The first mutation in a SDH-encoding gene (*SDHA*) was discovered in a patient presenting a devastating necrotizing encephalomyopathy affecting the central nervous system, known as the Leigh syndrome and characterized by bilateral damage of the basal ganglia and brain stem (Bourgeron et al., 1995). Leigh syndrome, a typical mitochondrial disease, is so far known to possibly results from mutations affecting over 30 genes either harbored by mtDNA or the nuclear genome (Scharfe et al., 2009). Five years later, in 2000, germ line mutations in *SDHD* was reported in families presenting with hereditary paraganglioma (PGL). PGL is characterized by benign, vascularized tumors in the head and neck, the most common tumor site being the carotid body, a chemoreceptive organ that senses oxygen levels in the blood. All SDH-encoding genes (*SDH A–D*) *plus SDHAF2* encoding a CII assembly factors, have been shown to potentially result in PGL (Burnichon et al., 2010), the *SDHB* mutations being associated with a high percentage of malignant paraganglionic tumors (Gimenez-Roqueplo et al., 2003) and with infrequent renal cell carcinoma (Williamson et al., 2014). A second illustration of the striking variability associated with mutations in nuclear genes encoding mitochondrial proteins is provided by mutations in *BCS1L*. This gene encodes a chaperone, member of the AAA family of ATPases that is necessary for the assembly of complex III in the mitochondria. First mutations in this gene were reported in 2001 in patients with complex III deficiency presenting tubulopathy, encephalopathy and liver failure (de Lonlay et al., 2001). One year later, additional mutations have been described in patients with a distinct phenotype, namely the GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death) syndrome, a recessively inherited lethal disease characterized by fetal growth retardation, lactic acidosis, aminoaciduria, cholestasis, and abnormalities in iron metabolism (Visapaa et al., 2002). Five years later, mutations were described in the very same gene in patients with the Björnstad syndrome, an autosomal recessive disorder associated with sensorineural hearing loss and *pili torti* (Hinson et al., 2007), bearing really no relationship with the previously described phenotypes associated with *BCS1L* mutations.

To account for the specific involvement of ear and hair tissues characteristic of the Björnstad syndrome, an exquisite sensitivity of these tissues to mitochondrial dysfunction was brought up. A similar hypothesis was raised in the early 90s but to account this time for the specific involvement of the retinas neurons in the Leber hereditary optic neuropathy (Wallace et al., 1988), a disease where the retinal ganglion cells are especially targeted. However, such proposal leaves aside the trivial observation that, similarly to the eyes, ear and hair tissues are far to be consistent involved in mitochondrial diseases, even if possibly involved (*e.g.* hair anomalies are observed with mutations in less than 10% of the genes known to cause mitochondrial disorders). Indeed, it is now clear that no simple explanation possibly accounts for the complexity of phenotypes associated with most mitochondrial diseases. The striking variability and complexity of the phenotypes associated with mitochondrial dysfunction most probably result from the multifaceted and tissue specific mitochondrial functions.

**Mitochondrial functions: ATP production, but…**

ATP synthesis by the respiratory chain (Fig. 3) is a well-recognized and prominent function of the mitochondria (estimated turn-over about 60 kg of ATP *per* day in humans) (Buono and Kolkhorst, 2001). However, under some instances at least, ATP synthesis by mitochondria is totally dispensable. Thus numerous types of cultured mammalian cells can be made Rho° (devoid of mitochondrial DNA) a condition where mitochondria are unable to synthetize their respiratory chain and the ATP synthetase (King and Attardi, 1989). These cells lacking ATP generation by their mitochondria grow quite happily provide glucose and pyruvate are made available to generate ATP, control redox status and/or peroxide levels. Without reaching such extreme condition (no mtDNA), it is reasonable to think that *in vivo* glycolysis can be substituted to mitochondria to ensure ATP synthesis at least partially and/or for a certain duration. In keeping with this, transient and/or partial hypoxia to which tissues can be faced will mechanically slow down respiratory chain activity due to oxygen shortage, a physiological event known to favor glycolysis. In the human body, fast glycolytic

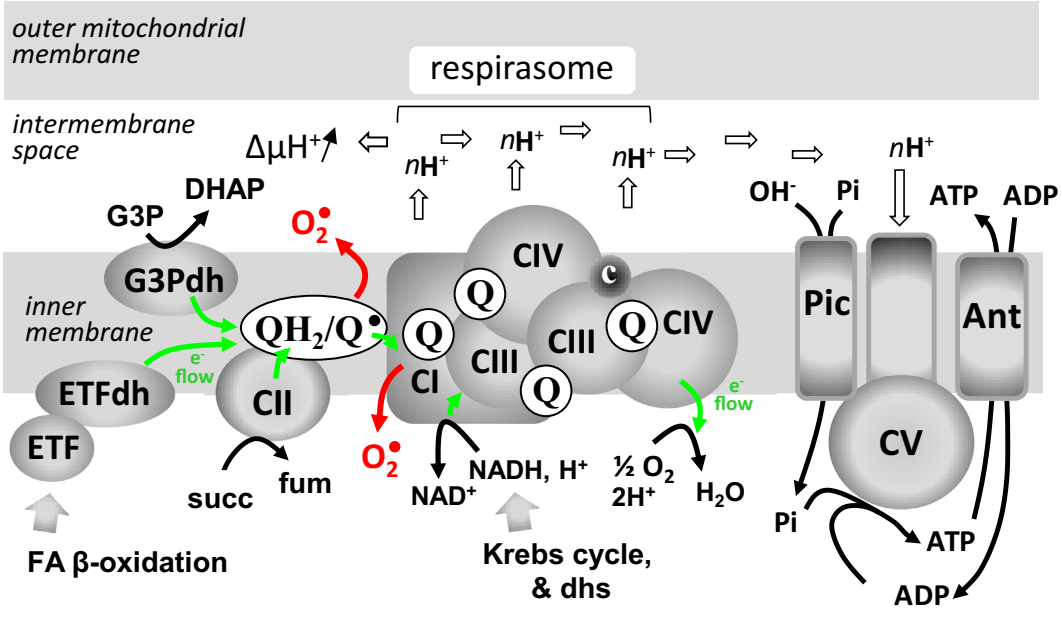


**Fig. 3.** A fragmentary scheme of the mitochondrial respiratory chain. Ubiquinone pools (Q) competitively reduced by several dehydrogenases, including Complex I (CI), Complex II (CII; succinate dehydrogenase; SDH), Electron-Transfering Flavoprotein dehydrogenase (ETFdh) or Glycerol-3-phosphate dehydrogenase (G3Pdh) feed electrons to the respirasome associating with several Complex III (CIII) and Complex IV (CIV). Simultaneously protons are extruded from the matrix space generating an electro-chemical gradient ($\Delta\mu H^+$) used by the ATPase (CV) to generate ATP from ADP and inorganic phosphate, respectively imported by the ATP/ADP translocase (ANT) and the Pi carrier (PiC). In response to various stimuli the ATPase switches from an energy-conserving to an energy-dissipating machinery; CV-dimers form a pore, known as the permeability transition pore (PTP), allowing the release of mitochondrial matrix components. When reduced ubiquinones ($QH_2$) accumulated upon over reduction by electron (green lines) semi-ubiquinones (Q°) are formed which readily react with oxygen to produce superoxide (in red). Additional abbreviations: dhs, dehydrogenases; FA, fatty acids; fum, fumarate; succ, succinate.

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muscle fibers — often termed white muscle due to low content in oxygen-binding myoglobin — are an example of such tissue where ATP is produced by glycolysis rather than by mitochondrial oxidative phosphorylation. Astrocyte glycolysis, a source of lactate for neurons, also appears the predominant pathway in these cells rather than mitochondrial OXPHOS (Pancani et al., 2011). Moreover, under a number of conditions, the use of mitochondrial activity is also not necessarily at its maximum: metabolic fluctuations linked to food absorption for example, or variable stimulation of skeletal muscle (exercise *versus* rest), are typical factors that will determine the extent of mobilization of the mitochondrial activity. Again, the study of mice deficient for PGC1-α and -β in the muscle revealed that endurance fatigue at low workloads is not limited by muscle mitochondrial capacity (Rowe et al., 2013). Indeed, mice lacking up to 60% of muscle oxidative capacity remain capable of normal locomotion, and indeed of voluntarily running up to 10 km per night! Only high workloads finally revealed a significant difference between control and deficient animals.

Assuming what was seen for the mouse is true for humans, despite surprisingly low requirement in mitochondrial ATP for skeletal muscle activity, myopathy is a frequent feature associated with mitochondrial disease (Scharfe et al., 2009). This suggests that alternative factors to ATP, dependent of mitochondrial activity, play a central role in disease expression and progression.

## The metabolic tangle

Mitochondria are crucial for both cellular catabolism and anabolism; each cell type, each tissue having its own metabolic chart. As a consequence, an impairment of mitochondrial function will presumably affect a huge number of metabolic pathways with considerable variations according to cell type, tissue and conditions. Indeed, some mitochondrial metabolic steps may be crucial in some cell type only. Thus the mitochondrial import of glutamate through the *GC1*-encoded carrier allowing glutamate oxidation through mitochondrial NAD-dependent dehydrogenase is in most cells reduced as compared to the transport through the glutamate–aspartate antiport of the malate–aspartate shuttle (Ramos et al., 2003). Yet mutation in *GC1* specifically causes early-onset neonatal epilepsies (Molinari et al., 2005) characterized by a typical electroencephalogram (EEG) pattern hallmarked by suppression burst (Ohtsuka et al., 1993; Vigevano and Bartuli, 2002). This can be ascribed to impaired glial cell functions in accordance with the fact that these cells, at variance with neurons, do not significantly express the glutamate–aspartate carriers (Ramos et al., 2003). Thus mitochondrial glutamate import in glial cells may be mostly dependent on GC1 glutamate carrier activity. This could explain particular glial cells involvement and the specificity of the brain pathology (Molinari et al., 2005).

Much other tissue specialization relies on specific contribution to synthesizing, breaking-down, and recycling of molecules. Many of these metabolic pathways directly or indirectly involve mitochondria and might be therefore affected when mitochondrial dysfunction occurs. The synthesis of different hormones, metabolism of fat, of sugar, of neurotransmitters, and many other pathways can be affected which echoes the number of clinical entities and phenotypic features identified in patients harboring mutations in gene encoding mitochondrial components (Table 1) (Scharfe et al., 2009).

The study of mitochondrial diseases has also revealed unexpected role for supposedly well-known mitochondrial components. Thus the study of *SDH* and *FH* mutant tumors has shown that mitochondrial metabolites play a crucial role in gene replication (Letouze et al., 2013). Studying these tumors has revealed that the balance of tricarboxylic acid cycle (TCAC) intermediates, namely α-ketoglutarate, succinate and fumarate, directly regulates a series of key cellular methylases controlling DNA and histone methylation. This biased gene methylation occurring in tumors of patients with *SDH* and *FH* mutation most probably explains tumor formation (Letouze et al., 2013). More generally, it points to a central role of tricarboxylic acid balance in the epigenetic regulation of gene expression (Salminen et al., 2014), a balance tightly controlled by mitochondria.

## Oxygen handling, for good and for bad

Mitochondria use oxygen as a terminal acceptor for the respiratory chain, oxygen being mostly reduced to water. However a fraction of the reducing power ends up in superoxide radicals (Fig. 3). Under normal conditions, oxygen free radical production will be mainly proportional to the flux in the respiratory chain, reflecting the rate at which unstable radical species of the respiratory chain (mainly semi quinones) are produced and react with oxygen (Bleier and Drose, 2012). A number of redox active, electron carriers (e.g. quinone, flavin, and cytochrome *b*) can become auto oxidized when over reduced and thus prone to react with oxygen to produced superoxides. Redox active components are not only associated with the respiratory chain. Thus the α-ketoglutarate dehydrogenase (KGDH), the branched-chain α-ketoacid dehydrogenase (BCKDH), the pyruvate dehydrogenase (PDH) complexes as well as the glycerol 3-phosphate dehydrogenase have been shown to be potential sources of superoxides and hydrogen peroxides (Orr et al., 2012; Adam-Vizi and Tretter, 2013). This will be especially significant for the former enzymes maintained reduced in the presence of NADH when respiratory chain complex I is defective, possibly causing a confusion for the source of ROS in this case. Blockade of these α-ketoacid dehydrogenases under reduced conditions favor ROS production (Adam-Vizi and Tretter, 2013). However conversely, nature being well organized, ketoacids (*i.e.* pyruvic, α-ketoglutaric acid) that often accumulated in case of complex I deficiency are potent $H_2O_2$ scavengers (Andrae et al., 1985).

Under physiological conditions, a part of activated oxygen species is used as signaling molecules for cell proliferation and tissue differentiation (e.g. β cells in the pancreas) (Hoarau et al., 2014). These physiological roles are enabled by conversion of superoxides to less reactive, transportable oxygen species, in particular $H_2O_2$ (Reczek and Chandel, 2014). In keeping with this, mitochondria are equipped with a whole series of molecules (i.e. tocopherol, glutathione) and inducible antioxidant enzymes (i.e. superoxide dismutase, thioredoxine and glutathione metabolizing enzymes, heme oxygenase), targeted to both hydrophobic and hydrophilic compartments of the organelles. Noticeably, evidence of induction of these antioxidant systems is a clear indication of increased mitochondrial ROS production (Fridovich, 1995; Geromel et al., 2001). The induction of antioxidant enzymes is mostly transcriptionally regulated, with NF-κB and actin-Keap1-bound Nrf2 (nuclear factor erythroid 2-related factor 2) important in this regulation. In comparison, the controversial use of exogenous redox probes (Maghzal et al., 2012), needing to compete with natural antioxidant defenses, might sometimes reflect abnormal redox reactions favored by unusual redox conditions.

When released by the mitochondria, ROS in excess are potentially detrimental to numerous macromolecules, including lipids, proteins and nucleic acids (Richter et al., 1995). In addition to targeting exposed unsaturated bounds of these molecules, radical species will rapidly destabilize iron–sulfur clusters of a series of mitochondrial enzymes, as long as in the vicinity. In keeping with this, the iron–sulfur cluster of aconitases (mitochondrial and cytosolic isoforms) is especially sensitive to superoxides, and these enzymes act as a natural sensor for oxidative stress in the cells (Hentze and Kuhn, 1996). Oxygen and its activated species will also react with other important signaling molecules, such as nitric oxide. In keeping with this, it is noteworthy that the direct toxicity of nitric oxide is modest but is greatly enhanced by reacting with superoxide to form peroxynitrite ($ONOO^-$).

In large excess, radical species will cause cell death (Green et al., 2014). But at low levels, they might also insidiously affect cellular components, especially mitochondrial genetic material. With time this could contribute to a decrease in mitochondrial activity, and contribute to the aging process. This has been the rationale of the free radical theory of

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aging and its derivatives, initially developed in the 1950s by Harman. However, few experimental data actually support this prediction. The investigation of the mtDNA mutator mice with increased somatic mutations in mitochondrial DNA due to impaired exonuclease activity of DNA polymerase γ failed to reveal oxidative impairment or elevated reactive oxygen species despite an accelerated aging phenotype.

## Apoptosis: mitochondria at work

Cell death can be triggered by a number of different signals part of which are possibly related to mitochondria (Green and Kroemer, 2004). Lack of ATP, oxygen radical overflow, release of mitochondrial protein factors, all have been shown to be potent cell death inducing factors. Both necrosis — traumatic cell death — and apoptosis, a developmentally regulated process, can result from mitochondrial signals. In vertebrate cells, apoptosis with its characteristic features (cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation) can involve the release of mitochondria entrapped factors, *e.g.* cytochrome *c*, Smac/DIABLO, and Omi/HtrA2. Released cytochrome *c* binds to Apaf-1 to form the apoptosome. Simultaneously, released Smac/Diablo and Omi/HtrA2 free caspases 3/7 and 9, trapped to XIAP. These latter then join the apoptosome to trigger the apoptosis processes. Other mitochondrial proteins associated with the respiratory chain or its biogenesis, such as Grim-19, a complex I subunit, or AIF (apoptosis-inducing factor), or present in the mitochondrial matrix, such as EndoG, also acts as proapoptotic factors released upon opening of the permeability transition pore formed by ATPase dimers switching from an energy-conserving to an energy-dissipating machinery (Giorgio et al., 2013). Small molecules such as $NAD^+$, coenzyme A, and thiamine pyrophosphate, presumably simultaneously leave mitochondrial matrix upon opening of the large pore with yet unknown consequence on the overall apoptosis process (Green et al., 2014).

A number of mitochondrial disease phenotypes involve tissue atrophy a process that may reflect excessive apoptosis which in a healthy adult would be responsible for the death of about 50 to 70 billion cells per day (Elliott and Ravichandran, 2010). On the other hand, insufficient apoptosis may trigger uncontrolled cell proliferation and tumor formation as observed in a subset of mitochondrial disease (Rustin and Kroemer, 2007). The nature, the severity of mitochondrial dysfunction, the context where it occurs (tissue, genetic background) can all affect the balance between pro- and anti-apoptotic processes and participate in the complexity of phenotypes associated with this diseases.

## Mitochondria, partners in the cell calcium connection

Mitochondria are endowed with a high capacity calcium uniporter, recently characterized in humans (Davidson et al., 2009), which can modulate cytosolic Ca2 signals, mitochondrial ATP synthesis and cell death (Brookes et al., 2004). Variable activity of the uniport is observed among tissues. In sensitive tissue this may avoid an excessive import into the mitochondria resulting in mitochondrial overload or/and cytosolic depletion. Cytosolic calcium signaling largely depends on a cross-talk between plasma membrane and cytoplasmic organelles, endoplasmic reticulum and mitochondria. Mitochondrial involvement relies on the enzymes and transporters, and relative positioning of the organelles. Again this appears to differ among cell types, and within a given cell, a peculiar positioning of mitochondria can differentiate a sub-populations of these in subplasmalemmal calcium-rich microdomains (Alonso et al., 2006).

Inside mitochondria, calcium regulates a number of key mitochondrial enzymes, activating Krebs cycle enzymes, *e.g.* pyruvate dehydrogenase, $NAD^+$-dependent isocitrate dehydrogenase, and α-KGDH (Denton, 2009). It can also activate the mitochondrial nitric oxide synthase (mtNOS) which consecutive inhibition of the nitric oxide-sensitive cytochrome *c* oxidase (Traaseth et al., 2004). Major matrix calcium overload can enhance generation of reactive oxygen species, increase lipid peroxidation, opening of the permeability transition pore, ultimately leading to apoptosis. On the other hand, dysfunction of the respiratory chain, especially complex I, has been shown to potentially alter calcium homeostasis (Visch et al., 2006) and, whole genome expression, suggests that this is due to the reduced expression of selenoproteins linked to endoplasmic reticulum stress (Voets et al., 2012).

## The structure affects the function and vice-versa…

Active mitochondria are not unevenly distributed within the cells. They interact with the filamentous structures of the cells which direct their movement (Iqbal and Hood, 2014), their fusion/fission (Li et al., 2015). Mitochondria also interact with other organelles (*e.g.* endoplasmic reticulum (Lahiri et al., 2014)) which favors metabolic exchanges and signaling, or appear concentrated in cell territories when needed (*e.g.* perinuclear location during phase of cell division (Wakai et al., 2014), or synaptic extremities (Brusco and Haas, 2015)). Echoing the various requirement of each cell type, the mitochondrial network, resulting from a set a fusion and fission events, differs from one cell type to another. The spatial location, the more or less fused structure of the network is under the tight control of a number of genes. It is therefore not surprising that mutation in these genes can result in severe human diseases. For example, deleterious mutations in the *OPA1* gene, encoding a dynamin-related protein playing a role in the mitochondrial fusion, have been shown to be a frequent cause of dominant optic atrophy (Landes et al., 2010). The same protein has also been claimed to play a role in sporadic Parkinson's disease by acting on the structure of the mitochondrial network (Santos et al., 2014). Perturbation of the network might affect other crucial phenomena in cascade such as mitophagy supposed to ensure the elimination of defective mitochondria, or undesired ones. Accordingly, mutations in PINK1 and Parkin, both regulating mitophagy, are known to cause recessively inherited forms of Parkinson's disease (Han et al., 2014) and associated with cancer in a subset of the cases (Matsuda et al., 2015).

On the other hand, altering mitochondrial function by uncouplers, or inhibitors of the respiratory chain, or mitochondrial activity by varying glucose concentration used to culture cells, is known to readily affect the structure of the mitochondrial network (Liot et al., 2009; Plecita-Hlavata et al., 2008). This suggests that severe defects of the respiratory chain should as well result in detectable disturbance of the mitochondrial dynamics. However, if the network appears peculiarly sensitive to numerous stress conditions in cells (primary cultured fibroblasts) with a respiratory chain defect, mitochondrial dynamics is not frequently affected by mitochondrial dysfunction under basal conditions (Peng et al., 2012; Guillery et al., 2008). As a result, structure of the mitochondrial network can hardly be used as a hallmark of mitochondrial dysfunction.

## Mitochondria throughout life

With the generation of hazardous free radicals, the preferred targeting of many toxic compounds, the capacity to replicate and expand its exposed DNA, the mitochondrial machinery has in itself all what is needed for self-destruction. With time, dysfunction of these partially self-replicative organelles can theoretically affect a significant part of the mitochondrial population of the organism and exceed a threshold, specific to each cell type or tissue, above which the overall function of the organ is affected.

Such changes in mitochondrial population with time have been traced in stem cells from colorectal epithelium in more than control 200 individuals by studying mtDNA (Greaves et al., 2014). It was first observed that the rate of appearance of mtDNA point mutation does not increase with age and remains low. On the other hand, the load of clonally expanded mutation, originating from early or mid-life, increased to a point where with age, set of cells in the crypts of this

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epithelium were functionally deficient (Greaves et al., 2014). Similar patterns of clonally expanded somatic mtDNA mutations were observed in the colon of heterozygous mtDNA mutator mice and aging humans (Baines et al., 2014). Critical areas in other tissues, including skeletal muscle and neurons in the *substantia nigra* appear to undergo similar process of clonal expansion of mutant mtDNA (Khrapko and Turnbull, 2014). It also appeared that the time required for these somatic mtDNA mutations to significantly expand makes late-life appearing mutations less detrimental to the aged organisms than long standing ones originating from early life.

Incidentally, these observations in healthy individuals imply that cells harboring defective mitochondria escape death processes and that, within cells, defective mitochondria, at least part of them, escape mitophagy, the quality control of the cell organize to get rid of damaged mitochondria (Lemasters, 2005). Of course, the study of patients with mitochondrial diseases already indicates that at least in some cases, part of defective mitochondria can escape this quality control and that this burden is compatible with life for long period of time (Munnich and Rustin, 2001). In keeping with this, also being essentially homoplasmic, deleterious mutations of mtDNA associated with LHON have phenotypic consequences often relatively late in life (Pfeffer et al., 2013). Interestingly enough this also applies for defects originating from nuclear genes where all mitochondria are supposedly similarly affected (Munnich and Rustin, 2001). In this instances, other mechanisms than clonal expansion of a sub population of mitochondria has to be at work. In some instances, we could make the assumption that defective mitochondria do part of the work and that defect remain functionally largely silent under basal conditions (Rowe et al., 2013). This would allow escaping surveillance by adequate cellular systems. Then, under more requiring, or stressful conditions, mitochondria cannot meet the demand anymore, and a disease then reveals. Escaping the cellular surveillance gives the chance to defective mitochondria to survive, multiply and finally to take over functional mitochondria.

For mtDNA mutations expanded with age, hopefully transmission of the deleterious ones is extremely rare suggesting the occurrence of a purifying selection against mutant mtDNA harboring cells or mitochondria during human reproductive process (Stewart and Larsson, 2014). However recent finding on mice indicated that low levels of maternally inherited mtDNA mutations in non-mutated PolgA off spring of mtDNA mutator mice when present during development can affect both overall health and lifespan negatively (Ross et al., 2014).

## Finally the therapy…

A spectacular range of phenotypes and a frequently delayed onset are among the most puzzling observations that can be made in inherited mitochondrial diseases. Examination of the complex genetics of these diseases, possibly arising from mutation in either the mitochondria or the nuclear DNA, and of the many and crucial functions of mitochondria already provides some rationale to our lack of effective treatment. However, a number of additional factors can actually add to the difficulty. Above, were only considered inheritable mitochondrial diseases. As a matter of fact, mitochondria are also the target of a huge number of natural toxics and industrial pollutants present in our environment and to which each of us is variably exposed (Belyaeva et al., 2008; Arnold et al., 1985). Individual history also includes our genetic background that has been shown to largely affect the actual consequences of mitochondrial deficiency (Benit et al., 2010). Altogether these factors presumably explain why phenotypes vary so much between patients and are so difficult to understand. This also has direct consequence when attempting to fight these diseases, with therapeutic targets to be changed for each condition and possibly according to patients.

Over the last twenty years, proof-of-principle studies have shown success, sometimes spectacularly, to improve the biochemical defect in a number of mitochondrial diseases in cultured cells or even in laboratory animals. These studies either specifically targeted the gene mutation, especially mtDNA mutations (peptide nucleic acids (Taylor et al., 1997), zinc finger nuclease (Gammage et al., 2014), fragment of the tRNA synthase (Perli et al., 2014), allotopic expression of CI subunit (Ellouze et al., 2008)), their consequences (targeting oxidative stress by antioxidants (Geromel et al., 2002), amino acids imbalance (Garone et al., 2014), shift of energetic substrates by changing the food (Schiff et al., 2011)), or aimed at increasing oxidative capacity without correcting the genetic defect (increased mitochondrial biogenesis (Cerutti et al., 2014), PPAR ligands (Wenz et al., 2008)) or at bypassing respiratory chain blockades irrespective from their genetic origins using heterologous genes (*Ndi1* (Marella et al., 2010) and *AOX* (El-Khoury et al., 2013)). These studies are frequently reviewed and their potentialities appropriately emphasized (Russell and Turnbull, 2014).

In contrast, very few data have been published from clinical trials, and even less reporting clear-cut positive effect of any treatment. To date mostly single-case reports of successful therapy have been published and proposed medication (vitamins, antioxidants) is largely supportive (Pfeffer et al., 2012). This presumably originates from the difficulties to cope with the heterogeneity of these diseases (one gene different phenotypes), individual reaction to disease (severity and course) and treatment (responders and non-responders) and, in this context, to get sufficient cohorts to organize trials. A typical example of this is for coenzyme $Q_{10}$ therapy. Primary deficiency of coenzyme $Q_{10}$ biogenesis is a quite rare defect. Patients have a reduced respiratory chain activity that is readily restored to control value *in vitro* in cells (cultured fibroblasts, circulating lymphocytes) or isolated mitochondria (skeletal muscle) by coenzyme $Q_{10}$ (Rotig et al., 2000). Accordingly, in a subset of these patients with primary deficiency, providing the missing coenzyme by oral supplementation had a spectacular and long standing effect. In contrast, trialing coenzyme $Q_{10}$ in patients with various mitochondrial diseases, or even with coenzyme $Q_{10}$ depletion, resulted in either negative or ambiguous results, generally unpublished (Geromel et al., 2002). As a result, having no detectable toxicity, coenzyme $Q_{10}$ is given to a large number of patients with no real proof of efficacy according to gold standards still called by the community (Pfeffer et al., 2014).

This situation should be however greatly improved, along with other genetic diseases, as gene therapy is making regular progress. In particular, targeting isolated organs or tissues, *e.g.* the eyes, with vectorized adequate gene constructs is now in a foreseeable future. It has already shown some exciting promises for mitochondrial and non-mitochondrial eye diseases, respectively blindness due to *Aif* mutation (Lechauve et al., 2014) and to *CHM* mutation, namely choroideremia (Black et al., 2014). It should apply to mitochondrial diseases as well, and a trial for gene therapy using a nuclear version of the *ND4* gene in the case of Leber Hereditary Optic Neuropathy is about to start in France (Cwerman-Thibault et al., 2014).

Thus despite a number of unsolved questions and unavoidable difficulties associated with mitochondrial cytopathies, there are multiple strategies aiming at preventing or treating these diseases. Some of them, including organ specific gene therapy, are about to be tested in humans, and effective treatments should be available at least for a subset of these diseases in a reasonable future.

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